Preclinical evaluation of a semi-automated and rapid commercial electrophoresis assay for von Willebrand factor multimers

Pikta, Marika

2018-07

Pikta , M , Zemtsovskaja , G , Bautista , H , Nouadje , G , Szanto , T , Viigimaa , M & Banys , V 2018 , ' Preclinical evaluation of a semi-automated and rapid commercial electrophoresis assay for von Willebrand factor multimers ' , Journal of Clinical Laboratory Analysis , vol. 32 , no. 6 , 22416 . https://doi.org/10.1002/jcla.22416

http://hdl.handle.net/10138/304363
https://doi.org/10.1002/jcla.22416

unspecified
publishedVersion

Received: 1 December 2017 | Accepted: 31 January 2018

DOI: 10.1002/jcla.22416

RESEARCH ARTICLE

WILEY

Preclinical evaluation of a semi-automated and rapid commercial electrophoresis assay for von Willebrand factor multimers

Marika Pikta[1,2] | Galina Zemtsovskaja[1,2] | Hector Bautista[3] | Georges Nouadje[3] | Timea Szanto[4] | Margus Viigimaa[2] | Valdas Banys[5]

[1]North Estonia Medical Centre Laboratory, Tallinn, Estonia

[2]Department of Health Technologies, School of Information Technologies, Tallinn University of Technology, Tallinn, Estonia

[3]Sebia, Research and Developments Department, Parc Technologique Léonard de Vinci, Evry Cedex, France

[4]Coagulation Disorders Unit, HUSLAB Laboratory Services, Department of Hematology and Clinical Chemistry, Helsinki University Hospital, Helsinki, Finland

[5]Faculty of Medicine, Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Vilnius University, Vilnius, Lithuania

Correspondence
Marika Pikta, North Estonia Medical Centre Laboratory, Tallinn, Estonia.
Email: Marika.Pikta@regionaalhaigla.ee

Background: The von Willebrand factor (VWF) multimer test is required to correctly subtype qualitative type 2 von Willebrand disease (VWD). The current VWF multimer assays are difficult, nonstandardized, and time-consuming. The purpose of this study was to evaluate the clinical utility of the commercial VWF multimer kit by Sebia (Lisses, France), an electrophoresis technique yielding same-day results.

Methods: Ten healthy volunteer plasma samples, in-house reference plasma (IRP) and commercial normal plasma (CNP) samples, 10 plasma samples from patients with a known VWD type, 1 hemophilia A plasma sample, and 7 external quality assurance (EQA) samples were analyzed using the commercial VWF multimer kit. Additional coagulation testing included measurements of VWF antigen (VWF:Ag), VWF activity (VWF:Ac), and FVIII activity (FVIII:C).

Results: The CNP results revealed a relative loss of the highest molecular weight multimers; therefore, IRP was preferred as the reference sample. The interpretations of 10 patients with a known VWD type could be successfully reproduced and agreed with previous VWF multimer results. In all EQA surveys, the multimer results and final VWD diagnosis agreed with expert opinion.

Conclusions: The VWF multimer assay by Sebia is easy to perform and can be successfully implemented in any clinical laboratory for second-stage evaluation of VWD. The resolution power of multimer distribution is adequate to correctly classify VWD types 1, 2A, 2B, and 3.

KEYWORDS

electrophoresis, subtyping, von Willebrand disease, von Willebrand factor, von Willebrand factor multimer

1 | INTRODUCTION

von Willebrand factor (VWF) is a large multimeric adhesive sialoglycoprotein[1,2] that mediates platelet adhesion to sub-endothelium structures and acts as a factor VIII (FVIII) carrier molecule, thus stabilizing the procoagulant activity of FVIII in the circulation.[1-5] The protein is synthesized by endothelial cells and megakaryocytes[4,6-8] as a polypeptide and is composed of identical monomers that assemble into a series of multimers. The multimer organization is critical for the function of VWF.[4] Multimers may range in size from 500 kDa to >20 000 kDa[7] and are usually classified into categories according to the number of multimers (dimers) and size: low-molecular-weight (LMW, 1-5 dimers, 500-2500 kDa), intermediate-molecular-weight (IMW, 6-10 dimers, 3000-5000 kDa), high-molecular-weight (large) (HMW, 11-20 dimers, 5500-10 000 kDa), and ultra-high-molecular-weight (ultra-large) (UHMW, >20 dimers, up to 20 000 kDa) forms.[4,7]

UHMW multimers do not typically circulate in blood because of rapid proteolysis by the disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (known as ADAMTS13) that cleaves UHMW forms into smaller multimers soon after secretion.[4]

von Willebrand disease (VWD) is the most common congenital bleeding disorder, with a worldwide prevalence of 1%.[3,7,8] The current classification of VWD variants by the International Society on Thrombosis and Haemostasis (ISTH)[9] recognizes six different types, reviewed with updates elsewhere.[1,4,5,7] The diagnosis of VWD is aided by good correlation between the clinical picture and traditional (screening) assays, such as FVIII, VWF antigen (VWF:Ag), VWF activity (VWF:RCo or alternatively VWF:Ac),[3] and, in some cases, the collagen binding capacity of VWF (VWF:CB).[10,11] Classical screening assays are highly heterogeneous in terms of methodology and diagnostic efficacy[10]; thus, they may lead to over-, under- or misdiagnosis[7] and inadequate or inappropriate treatment of affected patients.[12] Therefore, additional confirmatory VWD tests, such as VWF multimers, are needed to distinguish type 2A and 2B VWD from type 2M (or type 1) VWD[12,13] and diagnose acquired VWD. However, multimer analysis is currently performed only by a limited number of expert laboratories because it is technically difficult, laborious, nonstandardized, and time-consuming.[14] Indeed, a high proportion of laboratories generate unreliable VWF multimer results[15-17] using in-house assays. To overcome technical difficulties and help in the standardization of the method, Sebia (Lisses, France) developed a simplified, same-day results semi-automated assay (Hydragel 5 von Willebrand multimers) to visualize VWF multimers. The purpose of our study was to evaluate the usefulness and fitness for clinical purpose of this newly available commercial agarose gel electrophoresis technique.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

Four targets for the evaluation of the VWF multimer pattern were selected as follows: (i) 10 healthy volunteer plasma samples; (ii) in-house reference plasma (IRP) samples and commercial lyophilized pooled normal plasma (CNP) samples (Diagnostica Stago S.A.S., Asnières sur Seine, France); (iii) 10 plasma samples from patients with known VWD type 1, type 2 (subtypes 2A, 2B, 2N), or type 3, among whom 9 patients were from Finland and 1 was from Estonia, plus one hemophilia A patient was selected; and (iv) 7 external quality assurance (EQA) samples from the "von Willebrand Factor parameters" survey provided by the ECAT Foundation (Voorschoten, the Netherlands).

The study was carried out at North Estonia Medical Centre in collaboration with Helsinki University Hospital, HUSLAB Laboratory, Coagulation Disorders Unit (in partnership with The Twinning Program of the World Federation of Hemophilia (WFH)). The study was performed according to the Declaration of Helsinki and was approved by two national ethical committees (both the Tallinn and Helsinki Ethical Committees on Medical Research).

2.2 | Coagulation assays

All plasma samples in question were analyzed for FVIII:C, VWF:Ag, and VWF:Ac using the STA-R Evolution analyzer (Diagnostica Stago S.A.S., Asnières sur Seine, France). The FVIII:C (STA Deficient VIII, STA PTT-A) and VWF:Ag (STA Liatest VWF:Ag) reagents were purchased from Diagnostica Stago S.A.S. (Asnières sur Seine, France). VWF:Ac was measured using the INNOVANCE VWF Ac reagent (Siemens Healthcare Diagnostics, Marburg, Germany) according to a previously described method.[18]

2.3 | Preparation of IRP

IRP was prepared from 10 healthy volunteer plasma samples. Blood specimens were collected into 3.2% sodium citrate tubes (BD Vacutainer, BD Diagnostics, Plymouth, UK), centrifuged at 1500 *g* for 10 minutes at room temperature to generate platelet-free plasma, pooled, aliquoted into Eppendorf type tubes (composed of a nonactivating plastic), and frozen at −70°C. Prior to testing, samples were thawed in a 37°C water bath (for approximately 5 minutes) and mixed thoroughly. The volunteers were healthy laboratory employees without history of hemorrhagic episodes, who were not taking any medication for at least 10 days before blood collection, had a normal coagulation screen profile and normal VWF screening assays results, and who provided written consent. Exclusion criteria consisted of a positive personal and/or family bleeding history, inflammation, pregnancy, and oral contraceptive use.

2.4 | VWF multimer method developed by Sebia

All constituents of the assay (reagents, instruments, and software) were provided by Sebia (Lisses, France). Plasma samples were treated with sample diluent (pH 5.0 ± 0.5) and were pre-incubated for 20 minutes at 45°C. The dilution ratio was adapted based on the VWF:Ag result according to the manufacturer's instructions. The treated plasma samples were grouped into quintuplets, loaded onto Hydragel 5 von Willebrand multimer gels, and then subjected to a migration step using a Hydrasys 2 system with the following parameters: under 1 W constant, 10°C, controlled by the Peltier effect, until 170 Vh has accumulated, and a duration of approximately 115 minutes. The multimers were fixed on the gel using rabbit origin anti-VWF antibodies and then were probed with a second-step immunofixation by horseradish peroxidase (HRP)-conjugated anti-mammalian IgG. VWF multimers were evaluated by visualization after coloring the gels with commercially available reagents (commercial abbreviation TTF1/TTF2), and densitometric gel scan/graphical curves were produced and visualized with Sebia Phoresis CORE software.

2.5 | Previous studies confirming the VWD diagnosis

Nine patients with VWD from Finland with a complex evaluation and follow-up at the European Haemophilia Comprehensive Care Center (EHCCC) in Helsinki were included. VWF multimers from 8 patients were

previously analyzed by SDS agarose electrophoresis, western blotting, and luminescent visualization recorded by photoimaging in 1 of 4 reference laboratories with long-standing expertise in VWD diagnostics: type 1 and type 2 VWD patients were analyzed at Lund University (Malmö, Sweden), Karolinska Institute (Stockholm, Sweden), or Finnish Red Cross (Helsinki, Finland), and type 3 patients were assessed in collaboration with Dr. R. Schneppenheim (Hamburg, Germany).[19] Genetic testing was performed (i) in type 2B and 2N to differentiate between platelet-type VWD and hemophilia A and (ii) in type 3 in connection with genetic counseling, and the results were consistent with the VWD types. The genotypes of all type 3 VWD patients have been previously reported.[19]

3 | RESULTS

3.1 | VWF multimers in normal samples

The Hydragel 5 von Willebrand multiyear agarose gels were used to run 4 test samples and one control (reference) sample simultaneously. Comparative analysis of the size spectrum and banding pattern of VWF multimers in IRP and CNP samples on gels and the quantitative results of IRP (LMW-M 15.9%, IMW-M 33.2% and HMW-M 50.9%) and CNP (LMW-M 24.6%, IMW-M 34.7% and HMW-M 40.7%) densitometric curves revealed a relative loss of the highest HMW multimers in CNP samples, probably due to the lyophilization process while preparing commercial plasma (Figure 1).[17,20] Thus, IRP was preferred as control (reference) plasma in further studies.

The qualitative visual assessment of the size and distribution of VWF multimers in the plasma samples of four healthy subjects resulted in the pattern depicted in Figure 2, where one band on the gel and one peak in the densitogram correspond to one multimer band. LMW multimers are located on the top of the gel and at the left side of the *x*-axis of the densitogram. Correspondingly, HMW multimers are located on the bottom of the gel and at the right side of the *x*-axis of the densitogram, and IMW multimers are located in-between. Although there is no consensus on the definition of the areas comprising LMW, IMW, and HMW multimers, for convenience in interpreting the results, the multimer bands of this quartet of healthy subjects were classified as follows: 1-3 left to right peaks in the densitogram would represent LMW multimers, peaks 4-7 would represent IMW multimers, and peaks 8 and onwards would represent the group of HMW multimers (Figure 2). The applied classification is specific to the Sebia method and differs from

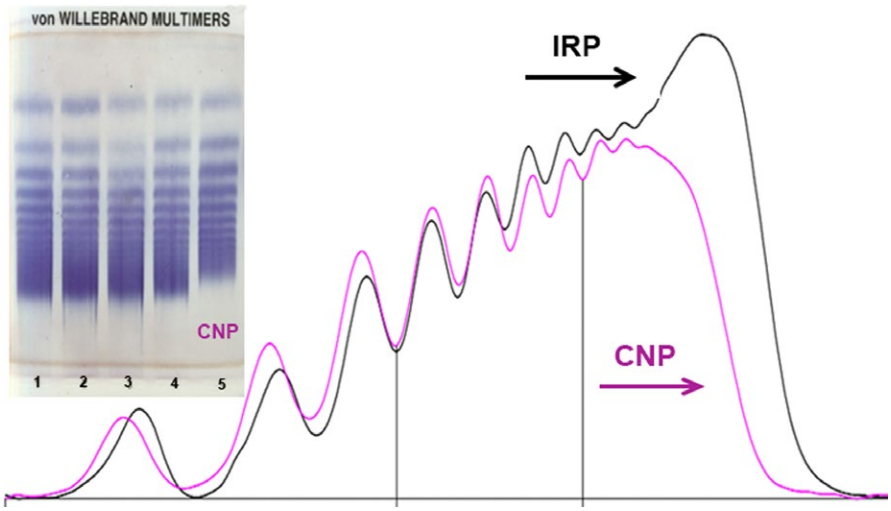

FIGURE 1 Electrophoresis gel of IRP samples (tracks 1-2), plasma samples from 2 randomly selected healthy individuals (tracks 3-4), and CNP samples (track 5). Densitograms of CNP and IRP

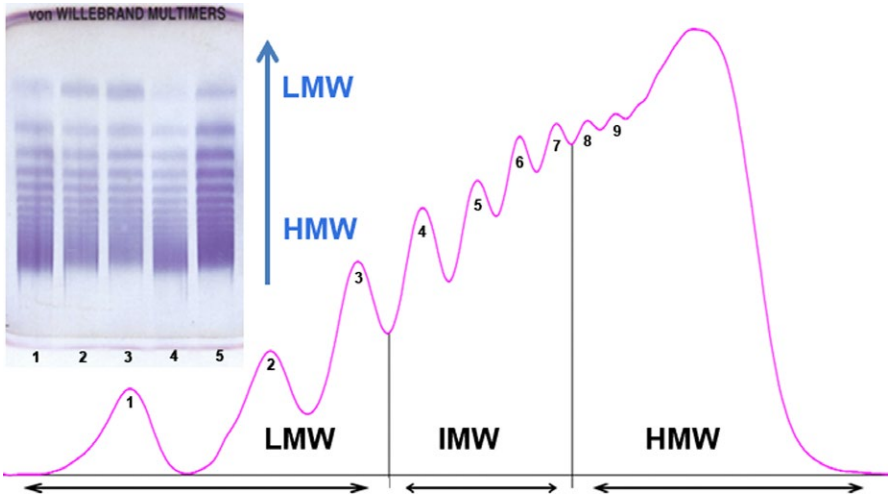

FIGURE 2 Electrophoresis gel of plasma samples from 4 different healthy individuals (tracks 1-4) and an IRP sample (track 5). Densitogram of IRP

reports in the literature (cited in the introduction), thus highlighting methodological differences between previously reported in-house and Sebia assays.

3.2 | VWF multimers in patient samples

VWF multimer electrophoresis interpretations of 9 VWD type (1, 2A, 2B, 2N, or 3) samples from HUSLAB, one type 2N VWD sample from Estonia, and one hemophilia A sample from Estonia could be successfully reproduced and were in agreement. The VWF:Ag, VWF:Ac, Ac/Ag ratio, FVIII:C results and VWF multimer pattern interpretations are summarized in Table 1. Several examples of the VWF multimer electrophoresis results of different VWD types are depicted in Figures 3-7. Three patients with type 1 VWD showed a normal VWF multimer pattern, although a relative decrease in the intensity of the multimer bands could be seen (Figure 3). As expected, the loss of HMW multimers was seen in both type 2A (Figure 4) and type 2B (Figure 5) VWD samples. Type 2N VWD patients exhibited normal multimeric patterns (Figure 6), and no signal was detected in two type 3 VWD patients (Figure 7), consequently leading to undetectable VWF multimer interpretation. The hemophilia A results were completely normal.

3.3 | VWF multimers in EQA samples

Throughout 2016 and 2017, the North Estonia Medical Centre Laboratory participated in seven EQA schemes of VWF modules, including VWF:Ag, VWF:Ac, FVIII:C, VWF multimers, and final conclusion (interpretation) (The ECAT Foundation, the Netherlands). In all 7 surveys, VWF multimer electrophoresis interpretation and final interpretation of the VWD type were in agreement with expert opinion (Table 2).

4 | DISCUSSION

VWF multimers should not be used as a standalone test to diagnose VWD.[21] The critical clinical utility of VWF multimers is in differentiating type 2A and 2B VWD from type 2M (or type 1) VWD,[12] as the correct classification of VWD is very important for the final diagnosis and treatment management.[22]

Unfortunately, until now, VWF multimer analysis has been performed only by a limited number of expert or reference laboratories (only 16%-18% of the participants of The ECAT Foundation EQA surveys[17]), mainly because the assay is technically complex, laborious, requires specially trained personnel, and is nonstandardized and time-consuming.[14,17,23] In addition, a certain proportion of interpretative errors arise due to test panels lacking the VWF multimer assay.[5,16]

The main methods in clinical use for the visualization of VWF multimers remain in-house-developed electrophoresis methods with typical overnight electrophoretic runs in agarose gels (alternatively, nitrocellulose or polyvinylidene difluoride) at concentrations ranging from 1% to 3%[14,21,23] with different options for immunologically detected multimer visualization (either radioactive, colorimetric, luminographic, or fluorometric methods).[23] Radioactive techniques are potentially hazardous, but conventional nonradioactive methods lack sensitivity and optimal resolution power,[13] potentially leading clinicians to misclassification of the VWD subtype.[20] Luminographic methods are much safer and are reported to allow visualization of multimers with confidence and high sensitivity.[13]

A very important issue is the turnaround time of VWF multimer analysis. The above-mentioned in-house methods are time-consuming, and although several of them have been somewhat optimized, they still require dozens of hours or even 3-4 days to complete.[13,20] In our case, the evaluated Sebia Hydragel 5 von Willebrand multimer electrophoresis assay produced same-day results in only 6 hours and 40 minutes. A significantly shorter turnaround time could encourage clinical laboratories to select such a method instead of the traditional, time-intensive procedures. Furthermore, in the case of analytical failure, same-day multimer analysis is more attractive because laboratories could repeat testing and release results quickly, although confirmation of the VWD type is not an urgent analysis.

Another disadvantage that many in-house electrophoresis methods possess is their inability to carry out quantitative analysis of VWF multimers.[13,20] Quantitative results can provide objective measures of the VWF structure to better define subtle changes in the VWD subtypes, such as dominant VWD type 1/2E (IIE) due to mutations in the D3 domain with aberrant triplet structure or the lack of outer bands or pronounced inner bands together with a relative decrease in LMW multimers.[24,25] However, the current classification for VWD does not consider the quantity of loss of the HMW multimers.[20] The Sebia method provides quantitative VWF multimer results and allows, if desired, splitting curves into multimer subsets. Laboratories may be able to establish normal ranges for different multimer sizes (LMW, IMW, and HMW) and quantify the percentage of loss in abnormal samples. The quantitative performance of the Sebia VWF multimer assay (reference ranges, clinical decision limits) should be assessed in future studies. Unfortunately, the Sebia method does not allow the visualization of VWF multimer triplets. Therefore, the main difference of it, compared with noncommercial assays, is the "quantification" itself, although this can be equally addressed by the in-house methods equipped with densitometers and associated software. Whichever method is in use, difficult cases, when increased subbands or abnormal triplet structures are observed, should undergo consultation with expert laboratories.

Other assays have been proposed in the literature—for example, direct biophysical fluorescence correlation spectroscopy—suggesting the quantitative nature of the method, short analysis time, and potentially low cost per sample.[14] However, such a method is not widely available for clinical laboratories, reinforcing the need for a rapid and commercially available VWF multimer method.

Currently, VWF multimer analysis demonstrates a relatively high error rate,[6] mainly reflected by the rather complex nature of the available methodologies. The ECAT Foundation collected data

TABLE 1 Summary of the VWF multimer and other coagulation results from patients with a known VWD type, previously examined by in-house VWF multimer assays or confirmed genetically

							Sebia VWF multimer			
								Quantitative results, %		
Patient No.	VWD type	VWF:Ag, %	VWF:Ac, %	VWF:Ac/VWF:Ag	FVIII:C, %	In-house VWF multimer interpretation[a]	Interpretation	LMW-M	IMW-M	HMW-M
1	1	32	34	1.06	52	Normal pattern	Normal pattern	9.0	15.7	75.3
2	1	23	22	0.96	59	Normal pattern	Normal pattern	14.3	28.9	56.8
3	1	51	42	0.82	57	Normal pattern	Normal pattern	12.5	32.0	55.5
4	2A	21	10	0.48	36	Loss of HMW multimer, abnormal triplet structure	Loss of HMW multimers	49.5	32.9	17.6
5	2B	126	51	0.40	70	Loss of HMW multimer, abnormal triplet structure	Loss of HMW multimers	74.8	21.6	3.6
6	2B	24	26	1.08	42	No data: type confirmed genetically	Loss of HMW multimers	55.8	35.2	9.0
7	2N	29	17	0.59	19	Normal pattern, decreased level of bands	Normal pattern	32.1	29.6	38.3
8	2N	58	71	1.22	33	No data: type confirmed genetically (see section 2.5)	Normal pattern	16.7	25.2	58.1
9	3	<5	<4	N/A	2	Undetectable	Undetectable	Quantitative results unavailable		
10	3	<5	<4	N/A	2	Undetectable	Undetectable	Quantitative results unavailable		
11	Not VWD (hemophilia A)	87	77	0.89	2	No data: type confirmed genetically	Normal pattern	18.3	30.6	51.1

[a]Results of former analysis of VWF multimer in patient samples—results were not known until analyzed by Sebia method.

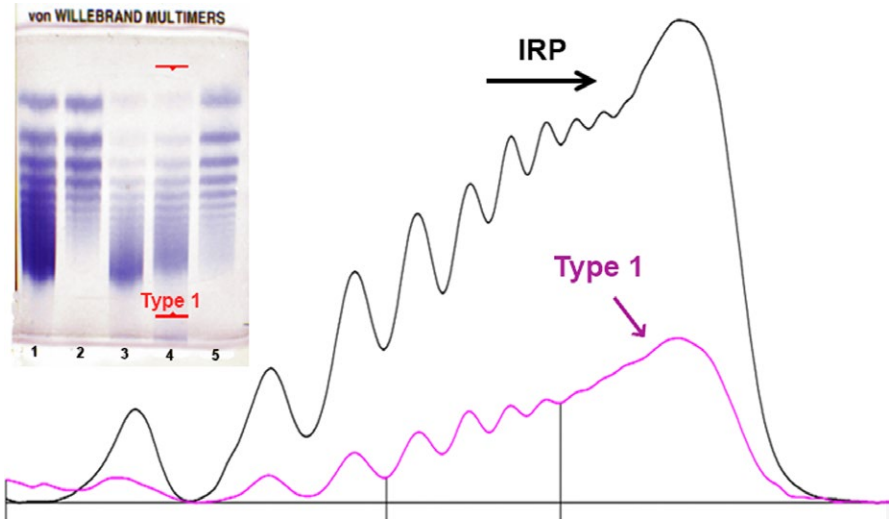

FIGURE 3 Electrophoresis gel of IRP (track 1) and type 1 VWD patient plasma (track 4). Densitograms: IRP vs type 1 VWD patient plasma

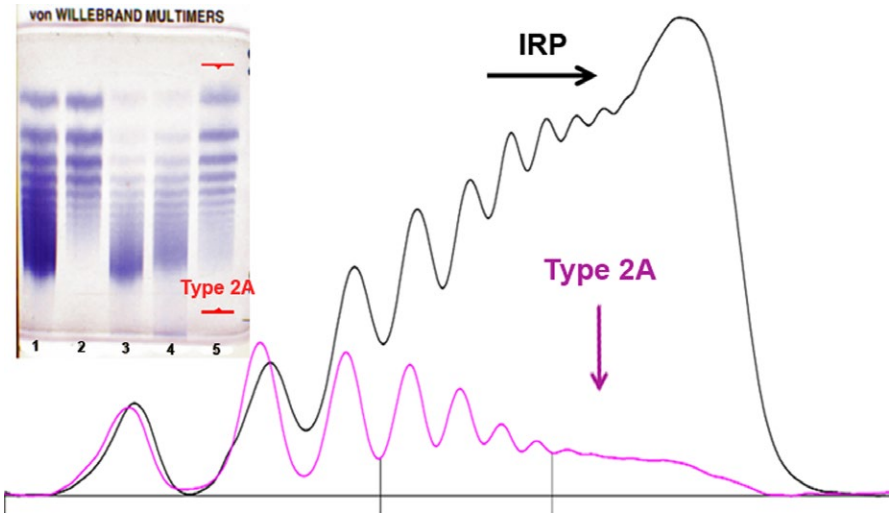

FIGURE 4 Electrophoresis gel of IRP (track 1) and type 2A VWD patient plasma (track 5). Densitograms: IRP vs type 2A VWD patient plasma

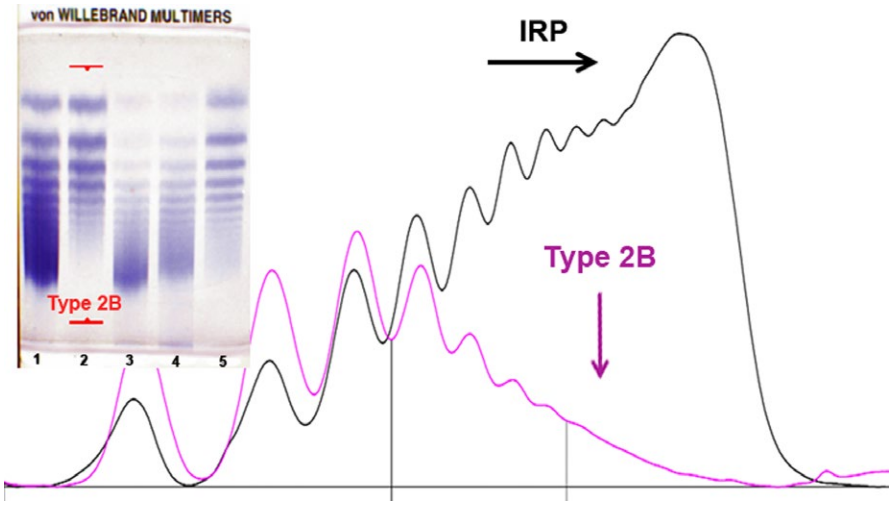

FIGURE 5 Electrophoresis gel of IRP (track 1) and type 2B VWD patient plasma (track 2). Densitograms: IRP vs type 2B VWD patient plasma

showing substantial error rates ranging from 10% to 52%.[17] The North American Specialized Coagulation Laboratory Association (NASCOLA) showed an overall 14.7% (7-22%) erroneous survey response rate from laboratories performing in-house VWF multimer analysis.[15] In our case, all EQA samples (including type 1 and type 2A VWD patients) were correctly visualized and interpreted by the new commercial VWF multimer assay, demonstrating its reliability.

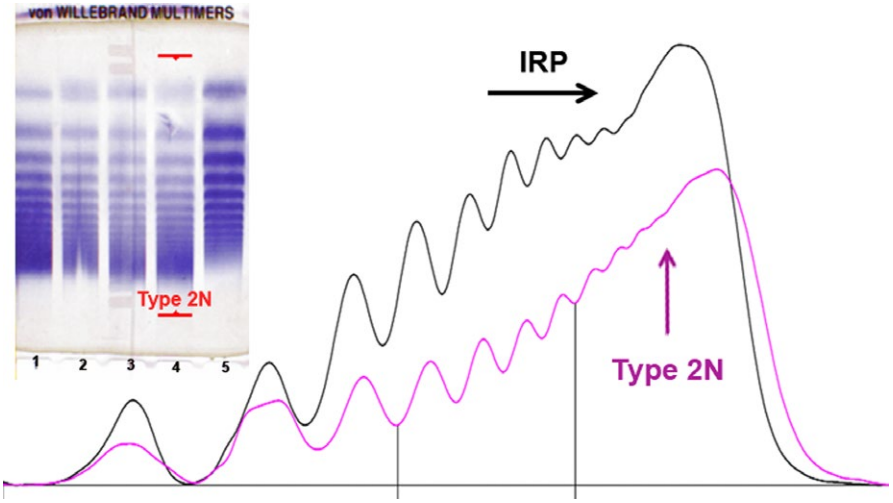

FIGURE 6 Electrophoresis gel of IRP (track 1) and type 2N VWD patient plasma (track 4). Densitograms: IRP vs type 2N VWD patient plasma

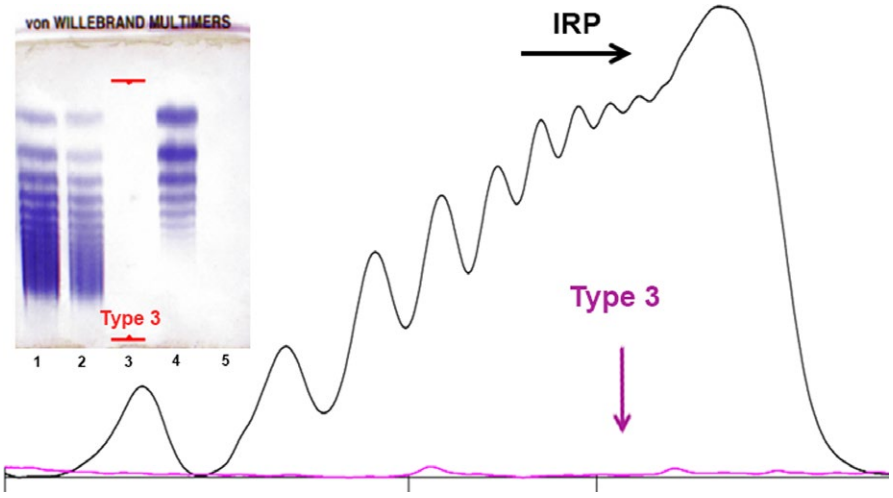

FIGURE 7 Electrophoresis gel of IRP (track 1) and type 3 VWD patient plasma (track 3). Densitograms: IRP vs type 3 VWD patient plasma

TABLE 2 Summary of VWF multimer analysis in EQA samples. Reproduced with permission from The ECAT Foundation (the Netherlands)

		Sebia VWF multimer				
			Quantitative results, %			
EQA survey No.	EQA sample	Interpretation	LMW-M	IMW-M	HMW-M	Conclusion on VWD type
2016-1	Normal control plasma	Normal distribution	23.3	33.1	43.6	Not VWD
2016-2	Type 1 VWD patient	Normal distribution	18.3	30.0	51.7	Type 1 VWD
2016-3	Type 2 VWD patient	Lack of IMW-M and HMW-M	73.8	11.5	14.7	Type 2A VWD
2016-4	Type 1 VWD patient	Normal distribution	10.7	26.8	62.5	Type 1 VWD
2017-1	Normal control plasma	Normal distribution	26.9	31.9	41.2	Not VWD
2017-2	Type 2 VWD patient	Lack of IMW-M and HMW-M	84.0	11.3	4.7	Type 2A VWD
2017-3	Type 1 VWD patient	Normal distribution	24.7	33.0	42.3	Type 1 VWD

Another question is whether the VWF multimer assay is needed in the VWD testing panel if VWF:RCo (alternatively VWF:Ac) and VWF:CB to VWF:Ag ratios are used as surrogate markers for the loss of HMW multimers.[7,10,11,16] The UK Haemophilia Center Doctors Organization guideline approved by the British Committee for Standards in Haematology recommends that such ratios be used to distinguish between types 2A and 2M (evidence level 1B).[21] A VWF:RCo/VWF:Ag ratio <0.7 should lead clinicians to look for type 2 VWD with a qualitative VWF defect and not type 1 VWD. However, the technical limitations of most VWF:RCo assays used

in laboratories worldwide make the VWF:RCo/VWF:Ag ratio unreliable, especially at the levels of VWF:Ag less than 15-20 IU/dL (%).[9] In such cases, the VWF multimer assay is very helpful to confirm or neglect the evidence of the loss of HMW multimers.[26]

Recently, the Sebia method was extensively evaluated by other authors, who provided positive comments on the fundamental consistency of the obtained data and presented reports at international meetings.[27] The same group of scientists additionally published a chapter on VWF multimers in the book available from Springer Science + Business Media (Hemostasis and Thrombosis: Methods and Protocols, Methods in Molecular Biology, vol. 1646). This paper describes the multimer methodology developed by Sebia in detail.[28]

5 | CONCLUSIONS

The new commercial VWF multimer assay (Hydragel 5 von Willebrand multimers; Sebia, Lisses, France) may represent a good alternative to traditional in-house assays. The Sebia method is easy to perform and can be successfully implemented in any clinical laboratory for second-stage evaluation of VWD. This method is a semi-automated agarose gel electrophoresis assay with ready-to-use gel and reagents, simple to carry out, and rapid (same-day results) compared with other (mainly in-house) methods. Visualization of the multimer distribution and densitometric analysis, together with the applied LMW, IMW, and HMW multimer classification, provide adequate resolution to correctly classify types 1, 2A, 2B, and 3 VWD cases. This new assay can be processed in routine use on a classical Sebia Hydrasys 2 multiparameter instrument. Furthermore, there is no need for additional training of laboratory technicians, and all of the main steps and instrument software are easily understandable and operated in a similar manner as other Sebia electrophoresis techniques (ie, serum/urine protein electrophoresis, immunofixation). Only the interpretation of the results should be carried out by or in consultation with experts. Nevertheless, the utility and value of this commercial method as an alternative for in-house assays must still be confirmed in future analyses. Evidence should be collected by the EQA organizers who have a substantial amount of data on the available methods. Likewise, larger-scale methods and comparison studies should be carried out because the small number of patients in our study was a major limitation.

ACKNOWLEDGMENTS

Research funding: Sebia (Lisses, France) donated the Hydragel 5 von Willebrand multimers kits. Karin Vilemson, a former employee of Mediq Estonia OÜ (distributor of Sebia in Estonia), consulted the authors on the study design, analysis, and interpretation of the data. The authors would like to express gratitude to laboratory technicians Galina Trofimova and Tatjana Tverskaja for excellent technical assistance related to sample testing.

CONFLICT OF INTERESTS

HB and GN are employees of Sebia (Research and Developments Department). MP, GZ, TS, MV, and VB have nothing to declare.

AUTHOR'S CONTRIBUTION

Marika Pikta (MP), Valdas Banys (VB), Margus Viigimaa (MV), and Galina Zemtsovskaja (GZ) designed the study. MP was responsible for recruiting the healthy volunteers and supervised the study. Timea Szanto (TS) provided samples for comparison. MP and GZ supervised the measurements. MP, VB, TS, and GZ interpreted the data. Hector Bautista (HB) and Georges Nouadje (GN) consulted authors in the process of writing the methods section of the report. MP, VB, and TS were the main contributors to the writing of the manuscript. All authors have accepted responsibility for the entire content of this submitted manuscript and have approved its submission.

ORCID

Marika Pikta http://orcid.org/0000-0002-5891-7589

Valdas Banys http://orcid.org/0000-0002-3020-7665

REFERENCES

1. De Meyer SF, Deckmyn H, Vanhoorelbeke K. von Willebrand factor to the rescue. *Blood*. 2009;113:5049-5057.
2. Millar CM, Brown SA. Oligosaccharide structures of von Willebrand factor and their potential role in von Willebrand disease. *Blood Rev*. 2006;20:83-92.
3. Roberts JC, Flood VH. Laboratory diagnosis of von Willebrand disease. *Int J Lab Hematol*. 2015;37(Suppl):11-17.
4. Stockschlaeder M, Schneppenheim R, Budde U. Update on von Willebrand factor multimers: focus on high-molecular-weight multimers and their role in hemostasis. *Blood Coag Fibrinol*. 2014;25:206-216.
5. Favaloro EJ. Rethinking the diagnosis of von Willebrand disease. *Thromb Res*. 2011;127(Suppl):S17-S21.
6. De Jong A, Eikenboom J. Developments in the diagnostic procedures for von Willebrand disease. *J Thromb Haemost*. 2016;14:449-460.
7. Favaloro EJ, Pasalic L, Curnow J. Laboratory tests used to help diagnose von Willebrand disease: an update. *Pathology*. 2016;48:303-318.
8. Holmberg L, Nilsson IM. von Willebrand's disease. *Eur J Haematol*. 1992;48:127-141.
9. Sadler JE, Budde U, Eikenboom JCJ, et al.; the Working Party on von Willebrand Disease Classification. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost*. 2006;4:2103-2114.
10. Favaloro EJ, Mohammed S. Evaluation of a von Willebrand factor three test panel and chemiluminescent-based assay system for identification of, and therapy monitoring in, von Willebrand disease. *Thromb Res*. 2016;141:202-211.
11. Meiring M, Badenhorst PN, Kelderman M. Performance and utility of a cost-effective collagen-binding assay for the laboratory diagnosis of von Willebrand disease. *Clin Chem Lab Med*. 2007;45:1068-1072.
12. Flood VH. Perils, problems, and progress in laboratory diagnosis of von Willebrand disease. *Semin Thromb Hemost*. 2014;40:41-48.

13. Budde U, Pieconka A, Will K, Schneppenheim R. Laboratory Testing for von Willebrand Disease: Contribution of Multimer Analysis to Diagnosis and Classification. *Semin Thromb Hemost*. 2006;32:514-521.
14. Torres R, Genzen JR, Levene MJ. Clinical Measurement of von Willebrand Factor by Fluorescence Correlation Spectroscopy. *Clin Chem*. 2012;58:1010-1018.
15. Chandler WL, Peerschke EI, Castellone DD, Meijer P; NASCOLA Proficiency Testing Committee. Von Willebrand factor assay proficiency testing. The North American Specialized Coagulation Laboratory Association experience. *Am J Clin Pathol*. 2011;135:862-869.
16. Favaloro EJ, Bonar RA, Meiring M, et al. Evaluating errors in the laboratory identification of von Willebrand disease in the real world. *Thromb Res*. 2014;134:393-403.
17. Meijer P, Haverkate F. An external quality assessment program for von Willebrand factor laboratory analysis: an overview from the European concerted action on thrombosis and disabilities foundation. *Semin Thromb Hemost*. 2006;32:485-491.
18. Pikta M, Zolotareva V, Tõnne J, Viigimaa M, Banys V. Implementation and Verification of New VWF: Ac Assay System with Components from Different Manufacturers. *Laboratorine medicina*. 2016;72:185-188.
19. Jokela V, Lassila R, Szanto T, et al. Phenotypic and genotypic characterization of 10 Finnish patients with von Willebrand disease type 3: discovery of two main mutations. *Haemophilia*. 2013;19:e344-e348.
20. Pruthi RK, Daniels TM, Heit JA, Chen D, Owen WG, Nichols WL. Plasma von Willebrand factor multimer quantitative analysis by in-gel immunostaining and infrared fluorescent imaging. *Thromb Res*. 2010;126:543-549.
21. Laffan MA, Lester W, O'Donnell JS, et al. The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology. *Br J Haematol*. 2014;167:453-465.
22. Favaloro EJ, Bodó I, Israels SJ, Brown SA. von Willebrand disease and platelet disorders. *Haemophilia*. 2014;20(Suppl):59-64.
23. Ledford-Kraemer MR. Analysis of von Willebrand factor structure by multimer analysis. *Am J Hematol*. 2010;85:510-514.
24. Michiels JJ, Smejkal P, Penka M, et al. Diagnostic Differentiation of von Willebrand Disease Types 1 and 2 by von Willebrand Factor Multimer Analysis and DDAVP Challenge Test. *Clin Appl Thromb Hemost*. 2017;23:518-531. https://doi.org/10.1177/1076029616647157
25. Gadisseur A, Berneman Z, Schroyens W, Michiels JJ. Laboratory diagnosis of von Willebrand disease type 1/2E (2A subtype IIE), type 1 Vicenza and mild type 1 caused by mutations in the D3, D4, B1-B3 and C1-C2 domains of the von Willebrand factor gene. Role of von Willebrand factor multimers and the von Willebrand factor propeptide/antigen ratio. *Acta Haematol*. 2009;121:128-138.
26. von Lillicrap D. Willebrand disease: advances in pathogenetic understanding, diagnosis, and therapy. *Blood*. 2013;122:3735-3740.
27. Favaloro EJ, Oliver S. Evaluation of a new commercial von Willebrand factor multimer assay. *Haemophilia*. 2017;23:e373-e377.
28. Oliver S, Lau KKE, Chapman K, Favaloro EJ. Laboratory testing for von Willebrand factor multimers. *Methods Mol Biol*. 2017;1646:495-511.

How to cite this article: Pikta M, Zemtsovskaja G, Bautista H, et al. Preclinical evaluation of a semi-automated and rapid commercial electrophoresis assay for von Willebrand factor multimers. *J Clin Lab Anal*. 2018;32:e22416. https://doi.org/10.1002/jcla.22416